

THAT WHICH IS CLAIMED:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:
- 5 (a) a nucleotide sequence encoding a polypeptide having at least about 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5;
- (b) the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5;
- 10 (c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6;
- (d) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence set forth in (a), (b), or (c);
- 15 (e) an antisense nucleotide sequence corresponding to a nucleotide sequence set forth in (a), (b), (c), or (d).
2. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a seed polypeptide having at least about 80% sequence identity to the nucleotide sequence set forth in any one of: SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.
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3. An isolated nucleic acid molecule comprising the nucleotide sequence set forth in any one of: SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.
- 25 4. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence set forth in any one of: SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.
5. An isolated nucleic acid molecule comprising a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, or 5.
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6. An isolated nucleic acid molecule comprising an antisense nucleotide sequence corresponding to a nucleotide sequence set forth in any one of SEQ ID NOS: 1, 3, or 5.
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7. An expression cassette comprising a nucleic acid molecule of claim 1, wherein the nucleic acid is operably linked to a promoter that drives expression in a plant cell.
- 10 8. An expression cassette of claim 2, wherein the nucleic acid encodes a heterologous polypeptide.
9. An expression cassette of claim 2, wherein the promoter is selected from the group consisting of seed-preferred, constitutive, chemically regulatable, tissue-preferred, and developmentally regulated promoters.
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10. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence selected from the group consisting of:
- 20 (a) a nucleotide sequence encoding a polypeptide having at least about 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5;
- (b) the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5;
- 25 (c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6;
- (d) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence set forth in (a), (b), or (c);
- 30 (e) an antisense nucleotide sequence corresponding to a nucleotide sequence set forth in (a), (b), (c), or (d).

11. The plant of claim 10 wherein the plant is selected from the group consisting of maize, wheat, barley, rice, rye, oats, and sorghum.
  - 5 12. Transformed seed of the plant of claim 10.
  - 10 13. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide comprising a nucleotide sequence encoding a polypeptide having at least about 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.
  - 15 14. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence comprising: the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.
  - 20 15. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence comprising: a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.
  - 25 16. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence comprising: a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.

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17. A transformed plant comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence comprising: an antisense nucleotide sequence corresponding to a nucleotide sequence set forth in any one of SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.
  18. A transformed plant cell comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence selected from the group consisting of:
    - (a) a nucleotide sequence encoding a polypeptide having at least about 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5;
    - (b) the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5;
    - (c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6;
    - (d) a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence set forth in (a), (b), or (c);
    - (e) an antisense nucleotide sequence corresponding to a nucleotide sequence set forth in (a), (b), (c), or (d).
  19. Seed of the plant of claim 18.
  20. A transformed plant cell comprising in its genome at least one stably incorporated expression cassette comprising a nucleotide sequence operably linked to a promoter that drives expression in a plant cell, the nucleotide sequence comprising: a nucleotide sequence encoding a polypeptide having at

least about 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:5.

21. A genetically modified maize plant comprising in its genome a mutated  
5 endogenous coding region, wherein the mutation is due to the presence of a transposon and the coding region is selected from the group consisting of:  
(a) a maize 50 kD gamma-zein coding region, wherein the coding sequence of the maize 50 kD gamma-zein coding region corresponds to the nucleotide sequence set forth in SEQ ID NO:1;  
10 (b) a maize 16 kD gamma-zein coding region;  
(c) a maize 15 kD beta-zein coding region; and  
(d) a maize 27 kD gamma-zein coding region.
- 15 22. Genetically modified seed of the plant of claim 21.
23. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:  
15 (a) the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6; and  
20 (b) an amino acid sequence of a polypeptide having at least 80% sequence identity to the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.
- 25 24. A method for improving the quality of a cereal grain by decreasing the level of a seed protein in the cereal grain, the method comprising:  
(a) transposon tagging an endogenous coding region in a cereal grain plant, wherein the coding region is selected from the group consisting of:  
30 (i) a maize 50 kD gamma-zein coding region, wherein the coding sequence of the maize 50 kD gamma-zein coding region

- corresponds to the nucleotide sequence set forth in SEQ ID  
NO:1;
- (ii) a maize 16 kD gamma-zein coding region  
(iii) a maize 27 kD gamma-zein coding region,  
5 (iv) a maize 15 kD beta-zein coding region,  
(b) selecting for a plant with improved grain quality.
25. A plant or plant parts produced by the method of claim 24.
- 10 26. Grain produced by the method of claim 24.
27. The method of claim 24 wherein the improvement in the quality of the grain is  
the result of at least one of: an increase in digestibility, an increase in  
nutritional value, a decrease in anti-nutritional properties of the grain, or an  
increase in the efficiency of wet-milling.  
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28. The method of claim 24 wherein the improvement in the quality of the grain is  
the result of a reduced level of nitrogen excretion into the environment as a  
result of feeding the grain.  
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29. The method of claim 24 wherein said plant is selected from the group  
consisting of maize, wheat, barley, rice, rye, oats, and sorghum.  
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30. The method of claim 24 wherein the handling properties of the grain are not  
altered.  
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31. The method of claim 24 wherein the decrease in the level of at least one of the  
16 kD gamma-zein, the 27 kD gamma-zein, or the 50 kD gamma-zein proteins  
is at least about 20%.

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32. The method of claim 24 wherein the decrease in the level of at least one of the 16 kD gamma-zein, the 27 kD gamma-zein, or the 50 kD gamma-zein proteins is at least about 50%.
- 5      33. The method of claim 24 wherein the decrease in the level of at least one of the 16 kD gamma-zein, the 27 kD gamma-zein, or the 50 kD gamma-zein proteins is at least about 70%.
- 10     34. The method of claim 27 wherein the increase in the nutritional value of the grain is a result of an increase in the lysine content of the grain of at least about 15%.
- 15     35. The method of claim 27 wherein the increase in the nutritional value of the grain is a result of an increase in the lysine content of the grain of at least about 35%.
- 20     36. The method of claim 24 wherein the improvement in the quality of the cereal grain is due to increased starch extractability in wet milling and a reduced need for polluting chemicals in wet milling.
- 25     37. A method for improving the quality of a cereal grain comprising stably introducing into a plant cell means for decreasing the level of any one of:  
(a) the nucleotide sequence set forth in SEQ ID NO:1;  
(b) a nucleotide sequence encoding the maize 15 kD beta-zein protein;  
(c) a nucleotide sequence encoding the maize 16 kD gamma-zein protein;  
and  
(d) a nucleotide sequence encoding the maize 27 kD gamma-zein protein.
- 30     38. A method for improving the quality of a cereal grain, by co-suppression or antisense suppression of an endogenous plant gene, said method comprising:

- PCT/EP2013/062010
- (a) transforming a cereal grain plant with at least one expression cassette comprising a promoter that drives expression in a plant cell operably linked to a nucleotide sequence selected from the group consisting of:
- (i) the nucleotide sequence set forth in SEQ ID NO:1;
- (ii) a nucleotide sequence encoding the maize 15 kD beta-zein protein;
- (iii) a nucleotide sequence encoding the maize 16 kD gamma-zein protein;
- (iv) a nucleotide sequence encoding the maize 27 kD gamma-zein protein; and
- (b) selecting transformed plants with improved grain quality.
39. The method of claim 38 wherein the improvement in the quality of the grain is the result of at least one of: an increase in digestibility, an increase in nutritional value, an increase in the efficiency of wet milling, or a decrease in anti-nutritional properties of the grain.
40. The method of claim 38 wherein the improvement in the quality of the grain is the result of a reduced level of nitrogen excretion into the environment as a result of feeding the grain.
41. A plant or plant parts produced by the method of claim 38.
42. Grain produced by the method of claim 41.
43. A method for increasing the nutritional value of a cereal grain by reducing the level of at least one seed protein in a cereal grain, the method comprising:
- (a) transforming a cereal grain plant with at least one expression cassette comprising a promoter that drives expression in the plant cell operably linked to a nucleotide sequence selected from the group consisting of:

- PCT/US2014/035202
- (i) a nucleotide sequence encoding the maize 18 kD delta-zein protein;
  - (ii) a nucleotide sequence encoding an engineered protein hordothionin 12; and
  - 5 (iii) a nucleotide sequence encoding a polypeptide whose amino acid sequence is at least about 30% combined methionine and cysteine; and
  - (b) selecting for plants with increased nutritional value.
- 10 44. A plant or plant parts produced by the method of claim 43.
45. Grain produced by the method of claim 44.
- 15 46. The method of claim 43 wherein the increase in nutritional value is due to an increase in at least one of: methionine, cysteine, or lysine in the grain.
47. The method of claim 43 wherein the increase in nutritional value is due to a reduction in gamma-zein levels.
- 20 48. The method of claim 47 wherein the nucleotide sequence encodes the maize 18 kD delta-zein protein and the gamma-zein protein levels in the grain are reduced by at least about 60%.
49. The method of claim 47 wherein the nucleotide sequence encodes the maize 18 kD delta-zein protein and the gamma-zein protein levels in the grain are reduced by at least about 80%.
- 25 50. The method of claim 47 wherein the nucleotide sequence encodes the hordothionin 12 protein and the gamma-zein protein levels in the grain are reduced by at least about 60%.

51. The method of claim 47 wherein the nucleotide sequence encodes the hordothionin 12 protein and the gamma-zein protein levels in the grain are reduced by at least about 80%.

5 52. The method of claim 46 wherein the increase in the nutritional value of the grain is a result of an increase in the lysine content of the grain of at least about 15%.

10 53. The method of claim 46 wherein the increase in the nutritional value of the grain is a result of an increase in the lysine content of the grain of at least about 35%.

15 54. The method of claim 43 wherein the handling properties of the grain are not altered.

20 55. A method of screening for a particular phenotypic trait in a plant, wherein the trait is determined by the level of a particular polypeptide in the plant, the method comprising:  
(a) contacting the plant with an antibody that selectively binds the polypeptide selected from the group consisting of:  
(i) the amino acid sequence set forth in SEQ ID NO:2;  
(ii) the amino acid sequence set forth in SEQ ID NO:4; and  
(iii) the amino acid sequence set forth in SEQ ID NO:6; and  
(c) determining the amount of the antibody binding and, thus, the level of the polypeptide that is present to screen for a particular phenotypic trait.

25 56. The method of claim 55 wherein the results of the screening are used to determine breeding crosses.

30 57. A method for increasing the hardness of corn grain, the method comprising:

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- (a) transforming a corn plant cell with an expression cassette comprising a promoter that drives expression in a plant cell operably linked the nucleotide sequence set forth in SEQ ID NO:1;
- (b) growing the plant cell under plant forming conditions, and
- 5 (c) selecting for plants with increased grain hardness.
58. The method of claim 57 wherein the hardness of the corn grain is increased over a phenotype that previously possessed a soft kernel phenotype.
- 10 59. The method of claim 57 wherein the grain previously possessing a soft kernel phenotype comprises in its genome a stably incorporated expression cassette comprising a nucleotide sequence encoding the protein hordothionin-12.
- 15 60. A plant or plant parts produced by the method of claim 57.
61. Grain produced by the method of claim 57.
62. A method for improving the quality of corn grain, the method comprising:
- (a) transforming a corn plant cell with an expression cassette comprising a promoter that drives expression in a plant cell operably linked to a nucleotide sequence comprising: the nucleotide sequence set forth in either of SEQ ID NO:3 or SEQ ID NO:5;
- (b) growing the plant cell under plant forming conditions, and
- 20 (c) selecting plants with improved grain.
63. The method of claim 62 wherein the improvement in the quality of the grain is the result of at least one of: an increase in digestibility, an increase in nutritional value, an increase in the efficiency of wet milling, or a decrease in anti-nutritional properties of the grain.

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64. The method of claim 62 wherein the improvement in the quality of the grain is  
the result of a reduced level of nitrogen excretion into the environment as a  
result of feeding the grain.
- 5      65. A plant or plant parts produced by the method of claim 62.
66. Grain produced by the method of claim 62.
67. The method of claim 63 wherein the increase in nutritional value is due to an  
10     increase in the digestibility of the grain.
68. The method of claim 63 wherein the increase in nutritional value is due to an  
increase in at least one of the tryptophan, methionine, or cysteine by at least  
about 50%.
- 15     69. A method for decreasing the caloric value of a cereal grain by decreasing the  
level of a particular seed protein, the method comprising:  
      (a) transposon tagging an endogenous coding region in a cereal grain plant  
          wherein the coding region is selected from the group consisting of:  
             (i) an 18 kD alpha-globulin protein set forth in SEQ ID NO:3; and  
             (ii) the sequence set forth in SEQ ID NO:5; and  
      (b) selecting for plants with grain decreased in caloric value.
- 20     70. The method of claim 69 wherein the grain with reduced caloric value  
comprises a component of a diet food for human consumption.
- 25     71. A method for decreasing the caloric value of a cereal grain by co-suppression  
or antisense suppression of an endogenous plant coding region, the method  
comprising:

- (a) transforming a cereal grain plant with at least one expression cassette comprising a promoter that drives expression in the plant cell operably linked to a nucleotide sequence selected from the group consisting of:
    - (i) the nucleotide sequence set forth in either of SEQ ID NO:3 or SEQ ID NO:5;
    - (ii) an antisense nucleotide sequence corresponding to the nucleotide sequence set forth in either of SEQ ID NO:3 or SEQ ID NO:5; and
  - (b) selecting for plants with grain decreased in caloric value.

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72. The method of claim 71 wherein the grain with reduced caloric value comprises a component of a diet food for human consumption.

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73. A plant or plant parts produced by the method of claim 71.

74. The grain produced by the method of claim 71.

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75. A method for decreasing the caloric value of a cereal grain by over-expression of an endogenous plant coding region, the method comprising:

- (a) transforming a cereal grain plant with at least one expression cassette comprising a promoter that drives expression in the plant cell operably linked to a nucleotide sequence selected from the group consisting of:
    - (i) the nucleotide sequence set forth in SEQ ID NO:1,
    - (ii) a nucleotide sequence encoding the maize 16 kD gamma-zein protein; and
    - (iii) a nucleotide sequence encoding the maize 27 kD gamma-zein protein; and
  - (b) selecting for plants with grain decreased in caloric value.

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76. The grain produced by the method of claim 75.

77. The method of claim 75 wherein the grain with reduced caloric value comprises a component of a diet food for human consumption.
- 5      78. A plant or plant parts produced by the method of claim 75.
79. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10.

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